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Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues

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Abstract

Resveratrol (3,5,4′-*trans*-trihydroxystilbene) is a natural phytoalexin present in grapes and red wine, which possesses a variety of biological activities including antioxidative activity. To find more efficient antioxidants by structural modification, resveratrol analogues, that is, 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-*trans*-stilbene (4-HS) and 3,5-dihydroxy-*trans*-stilbene (3,5-DHS), were synthesized and their antioxidant activity studied for the free radical-induced peroxidation of rat liver microsomes in vitro. The peroxidation was initiated by either a water-soluble azo compound 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) or Fe^{2+}/ascorbate, and monitored by oxygen uptake and formation of thiobarbituric acid reactive substances (TBARS). It was found that all of these *trans*-stilbene derivatives are effective antioxidants against both AAPH- and iron-induced peroxidation of rat liver microsomes with an activity sequence of 3,4-DHS>4,4′-DHS>resveratrol>4-HS>3,5-DHS. The remarkably higher antioxidant activity of 3,4-DHS is discussed.

Keywords: Lipid peroxidation; Antioxidant; Resveratrol; Microsome; Structure/activity relationship

1. Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a naturally occurring phytoalexin present in grapes and many other plants. It was found recently that this compound possesses a variety of biological activities [1–9]. For instance, its presence at high level in red wine (0.1–15 mg/l) [1] has been suggested to be linked to the low incidence of heart diseases in some regions of France, the so-called "French paradox", that is, despite high fat intake, mortality from coronary heart disease is lower due to the regular drinking of wine [2]. In addition, resveratrol has been shown to possess cancer chemopreventive activity [3,4]. Therefore, the past several years have witnessed intense research devoted to the biological activity, especially the antioxidative activity, of this compound [4b,4c,5–9], since free radical-induced peroxidation of membrane lipids and oxidative damage of DNA have been considered to be associated with a wide variety of chronic health problems, such as cancer, atherosclerosis and aging [10–12], and gene transcription can be regulated by oxidants, antioxidants and other determinants of the intracellular redox state [13]. Resveratrol has been reported to be a good antioxidant against the peroxidation of low-density lipoprotein (LDL) [6] and liposomes [7], a potent inhibitor of lipoxygenase [8], and able to protect rat heart from ischaemia reperfusion injury [9]. As a part of our ongoing research project on antioxidative effects of natural antioxidants and their derivatives [14], we found recently that some resveratrol derivatives bearing an *ortho*-diphenoxyl functionality possess much higher antioxidative activity against free radical-induced peroxidation in membrane mimetic systems [15]. Therefore, it is desirable to see if the same structure/activity relationship is also valid in biological systems. We report herein an in vitro study on the antioxidative effect of resveratrol and related *trans*-stilbene analogues, that is, 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-*trans*-stilbene (4-HS) and 3,5-dihydroxy-*trans*-stilbene (3,5-DHS), on free radical-induced peroxidation of rat liver microsomes. Microsomes, especially smooth surfaced endoplasmic reticulum,

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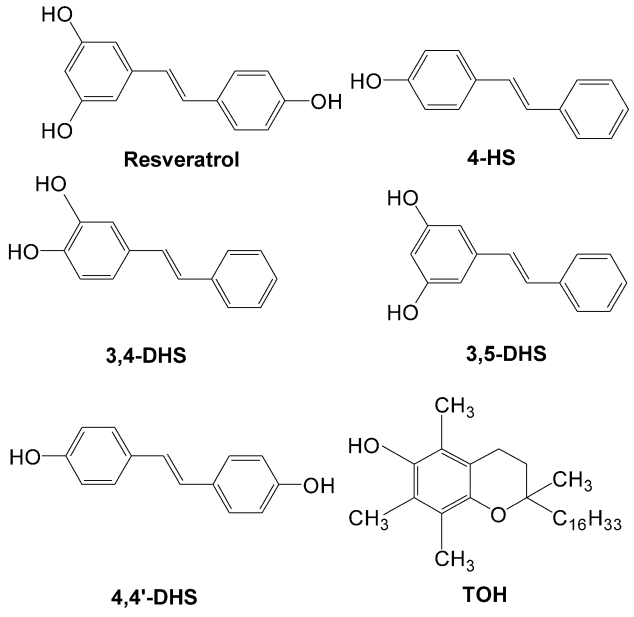
are particularly susceptible to oxidative stress because of the high content of polyunsaturated fatty acids, hence have been widely used as a model for oxidative stress and antioxidant studies [16]. The peroxidation was initiated by a water-soluble azo initiator 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH) and Fe^{2+}/ascorbate and measured by oxygen uptake and formation of thiobarbituric acid reactive substances (TBARS). It was found that resveratrol and its analogues, especially 3,4-DHS, are good antioxidants for both peroxy radical- and hydroxy radical-initiated peroxidation of rat liver microsomes.



2. Materials and methods

2.1. Materials

Resveratrol and its analogues 4-HS, 3,5-DHS, 4,4′-DHS and 3,4-DHS were synthesized with reference to the available method [17]. This procedure gave exclusively the *trans*-isomer and their structures were fully identified with ^{1}H NMR and EI-MS and the data are consistent with those reported in the literature [17]. The purity of the compounds was checked by HPLC. AAPH and butylated hydroxytoluene (BHT) were purchased from Aldrich. Thiobarbituric acid (TBA) was from Sigma. All other chemicals were of the highest quality available.

2.2. Preparation of rat liver microsomes

Female Wister rats weighing 250 ± 20 g were starved overnight before cervical dislocation. Liver microsomes were prepared by tissue homogenization with ice-cold 0.25 M sucrose–0.01 M Tris buffer, pH 7.4, with 1 mM EDTA (STE buffer), in a motor-driven glass homogenizer [18]. Microsomal fractions were isolated by removal of the nuclear faction at 8000 × *g* for 10 min and removal of mitochondrial fraction at 18,000 × *g* for 10 min. The microsomal fraction was sedimented in a Hitachi 55P-72 ultracentrifuge at 105,000 × *g* for 60 min, washed two times with 0.15 M KCl at 105,000 × *g* for 30 min. The membranes, suspended in 0.1 M potassium phosphate buffer, pH 7.5, were stored in a deep freezer maintained at − 20 °C. Microsomal protein was determined by the method of Lowry et al. [19].

2.3. Oxygen uptake measurements

The rate of oxygen uptake was measured in a closed glass vessel of ca. 2 ml in volume, thermostated at 37 ± 0.1 °C and provided with a magnetic stirrer, using a 5946-50 oxygen meter (Cole-Parmer Instruments, USA) which was able to record oxygen concentration as low as 10^{-8} M. Rat

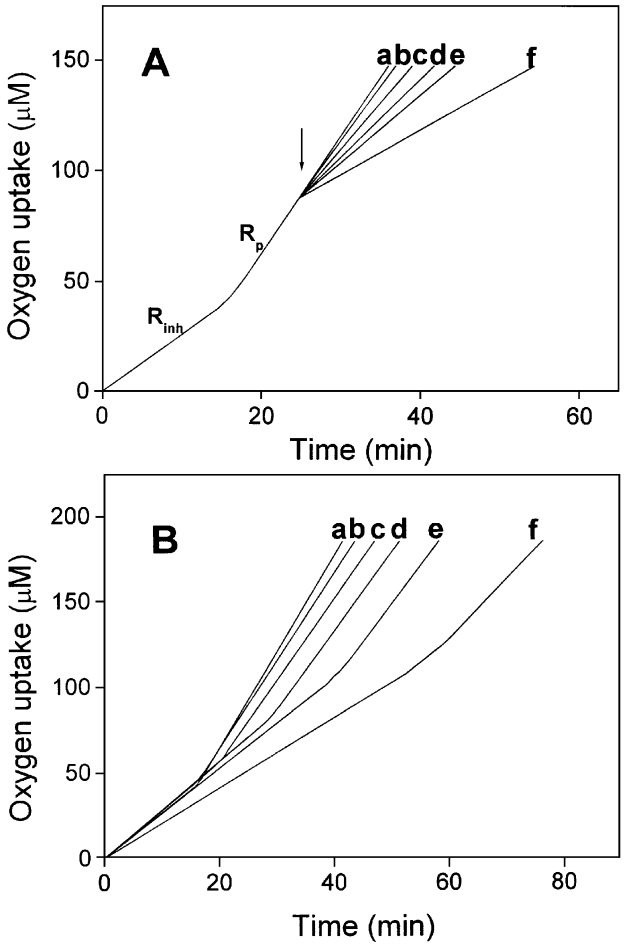


Fig. 1. Representative oxygen uptake curves recorded during the AAPH-induced and ROH-inhibited peroxidation of rat liver microsomes at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 2.0 mg/ml. $[AAPH]_0 = 8$ mM; $[ROHs]_0 = 5$ μM. (a) Native microsomes; (b) 3,5-DHS; (c) 4-HS; (d) resveratrol; (e) 4,4′-DHS; (f) 3,4-DHS. (A) The ROHs were added after the inhibition period and the arrow shows the position of adding the ROHs. (B) The ROHs were added before AAPH initiation.

liver microsomes were suspended in 0.1 M potassium phosphate buffer to the final concentration of 2.0 mg protein/ml. Resveratrol and its analogues (ROHs) were dissolved in DMSO to the concentration of 4.0 mM as stock solutions. The final concentration of DMSO in the suspension was less than 0.1% (v/v) that did not show appreciable interference to the reaction as evidenced by control experiments. AAPH was directly dissolved in the phosphate buffer (pH 7.5) and then injected into the suspension to initiate the peroxidation. Every experiment was repeated three times and values represent mean ± S.E. of the experiments.

2.4. Measurements of TBARS

The formation of TBARS was used to monitor lipid peroxidation [20]. Rat liver microsomes were incubated at 37 °C in 0.1 M potassium phosphate buffer, pH 7.5, and made up to a final protein concentration of 0.3–0.5 mg/ml. The peroxidation was initiated by either AAPH or Fe^{2+}/ascorbate and inhibited by ROHs which were added as an ethanol solution to the microsomal suspension and homogenized using a glass homogenizer. The final concentration of ethanol in the suspension was less than 0.5% v/v that did not show appreciable interference to the reaction as evidenced by control experiments. The reaction mixture was gently shaken at 37 °C and aliquots of the reaction mixture were taken out at specific intervals to which a TCA–TBA–HCl stock solution (15% w/v trichloroacetic acid; 0.375% w/v TBA; 0.25 N HCl) was added, together with 0.02% w/v BHT. This amount of BHT completely prevents the formation of any nonspecific TBARS, as well as preventing decomposition of AAPH during the subsequent boiling [21]. The solution was heated in a boiling water bath for 15 min. After cooling, the precipitate was removed by centrifugation. TBARS in the supernatant was determined at 532 nm using the extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ [20].

2.5. Determination of α-tocopherol (TOH)

TOH in rat liver microsomes was firstly extracted by hexane–ethanol partitioning (hexane:EtOH:microsomes = 12:3:1 v/v/v) as described previously [14c], which yielded >97% of TOH [22]. Then the TOH was separated by a Gilson model 702 liquid chromatograph with a Sychropack KPP-100 reversed-phase column (4 × 250 mm) and eluted with methanol–*iso*-propanol–formic acid (80:20:1 v/v/v) containing 50 mM of sodium perchlorate as a supporting electrolyte at a flow rate of 1 ml/min and determined electrochemically by using a Gilson Model 142 electrochemical detector by setting the oxidation potential at +700 mV.

3. Results

3.1. Inhibition of AAPH-induced microsomal peroxidation by ROHs as studied by oxygen uptake

Fig. 1 shows representative oxygen uptake curves recorded during the water-soluble azo initiator AAPH-induced rat liver microsomal peroxidation. In the absence of exogenous antioxidants, the oxygen uptake was still inhibited for about 16 min (Fig. 1A, line a), demonstrating the presence of endogenous antioxidants in the microsomes, for example, α − tocopherol, and ubiquinol-10 [12a], which can trap the initiating and/or propagating peroxyl radicals to inhibit the microsomal peroxidation. The oxygen uptake rate during the inhibition period is designated as R_{inh}. After the inhibition period, the oxygen uptake turned to faster, indicating depletion of the endogenous antioxidants. The turn-

Table 1
Kinetic parameters for the AAPH-induced peroxidation of rat liver microsomes and its inhibition by ROHs[a]

Antioxidant	R_{inh} (nM/s)	R_p (nM/s)	t_{inh} (min)	S.E. (%)	R_{inh}/R_p (%)	t_{inh} (AH)/t_{inh} (ROH + AH)
AH[b]	39.9 ± 0.6	82.1 ± 4.2	16.3 ± 0.3		48.6	
3,4-DHS[c]	29.8 ± 0.3				36.3	
4,4′-DHS[c]	43.3 ± 3.2				52.7	
Resveratrol[c]	55.9 ± 4.1				68.1	
3,5-DHS[c]	72.4 ± 2.5				88.2	
4-HS[c]	65.6 ± 1.2				79.9	
3,4-DHS[d]	34.8 ± 1.5	52.4 ± 0.6	53.7 ± 0.6	229.4		0.30
4,4′-DHS[d]	40.8 ± 1.7	71.6 ± 4.0	38.5 ± 2.3	136.2		0.42
Resveratrol[d]	44.8 ± 1.4	73.4 ± 3.9	27.8 ± 1.0	70.6		0.58
3,5-DHS[d]	44.6 ± 0.5	71.8 ± 4.5	17.5 ± 1.3	7.4		0.93
4-HS[d]	46.1 ± 3.0	70.0 ± 4.5	18.0 ± 1.7	10.4		0.90

[a] Peroxidation was monitored by oxygen uptake assay. The microsomes were suspended in 0.1 M phosphate buffer, pH 7.5, with protein concentration of 2.0 mg/ml; $[AAPH]_0 = 8$ mM. $[ROHs]_0 = 5$ μM. The data represent the means of three experiments.
[b] Endogenous antioxidants in rat liver microsomes.
[c] Added after depletion of the endogenous antioxidants.
[d] Added before the initiation.

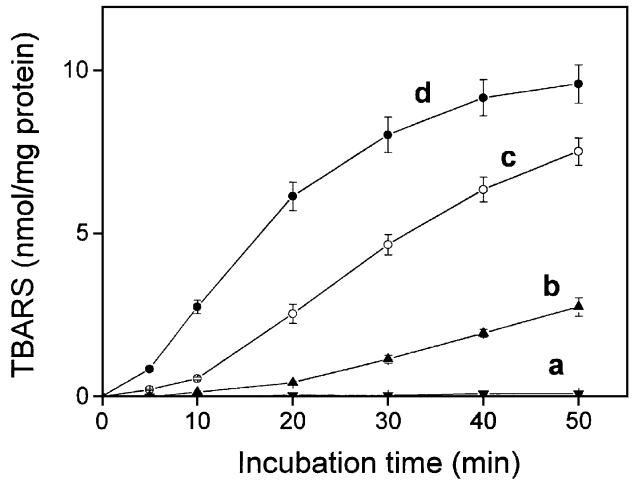


Fig. 2. Formation of TBARS during the AAPH-induced peroxidation of rat liver microsomes at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 0.5 mg/ml. The initial concentrations of AAPH were (a) 0, (b) 2, (c) 8 and (d) 20 μM, respectively. Values represent mean ± S.E. of three experiments.

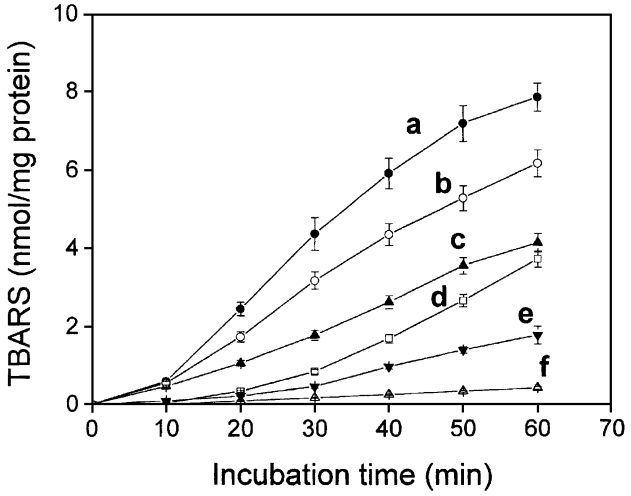


Fig. 4. Inhibition of TBARS formation during the AAPH-induced peroxidation of rat liver microsomes by ROHs at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 0.5 mg/ml. $[AAPH]_0 = 8$ mM; $[ROHs]_0 = 15$ μM. (a) Native microsomes; (b) 3,5-DHS; (c) 4-HS; (d) resveratrol; (e) 4,4′-DHS; (f) 3,4-DHS. Values represent mean ± S.E. of three experiments.

ing point from the inhibition period to the restoration of oxygen uptake refers to inhibition time, t_{inh}. The slope of the oxygen uptake curves after the inhibition period represents the intrinsic peroxidation rate, R_p, of the microsomes in the absence of antioxidants.

After a short time of the inhibition period, 5 μM resveratrol and its analogues (ROHs), that is, 3,4-DHS, 4,4′-DHS, 4-HS and 3,5-DHS, were added. It was found that all of these ROHs decreased the rate of oxygen uptake, but no inhibition period was observed (Fig. 1A, lines b–f). The kinetic behavior is similar to that of the AAPH-initiated peroxidation of linoleic acid in homogenous solution reported previously [23]. The decrease of the oxygen uptake rate follows the sequence of 3,4-DHS>4,4′-DHS>resveratrol>4-HS ~ 3,5-DHS. The kinetic data are listed in Table 1.

It is worth to note that although ROHs did not produce inhibition period when they were used alone (Fig. 1A, lines b–f), they increased remarkably the inhibition period of the native rat liver microsomes when the same amount of ROHs was added to the microsomal suspension before the initiation (Fig. 1B, lines b–f), revealing a synergistic antioxidant

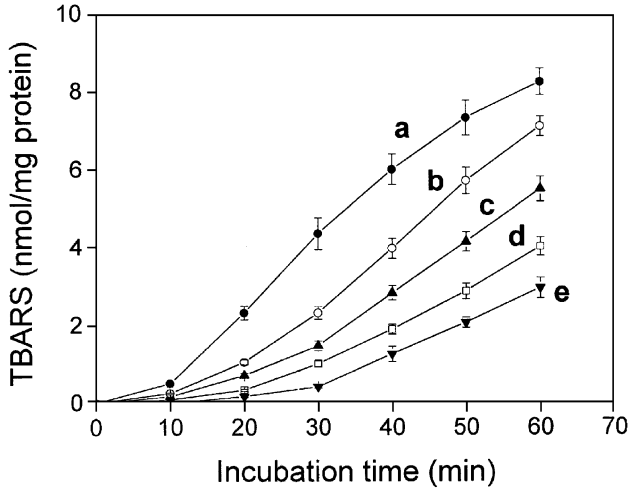


Fig. 3. Inhibition of TBARS formation during the AAPH-induced peroxidation of rat liver microsomes by resveratrol at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 0.5 mg/ml. The peroxidation was initiated with 8 mM of AAPH and the initial concentrations of resveratrol were (a) 0, (b) 5, (c) 10, (d) 15 and (e) 20 μM, respectively. Values represent mean ± S.E. of three experiments.

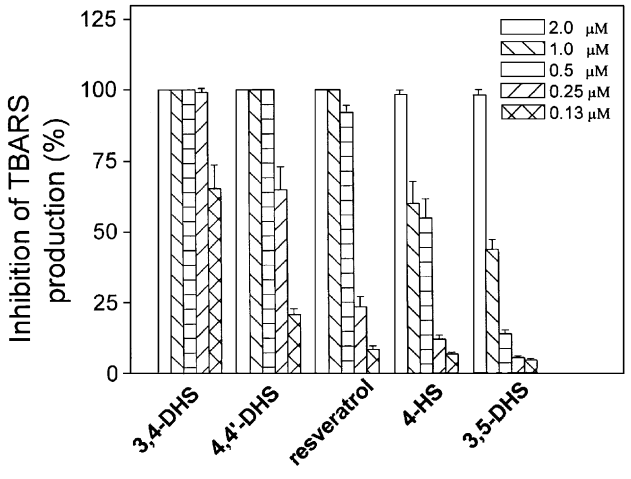


Fig. 5. Inhibition of TBARS formation during the Fe^{2+}/ascorbate-induced peroxidation of rat liver microsomes by ROHs at 37 °C. The rat liver microsomes were incubated in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 0.3 mg/ml for 30 min. $[FeSO_4] = 10$ μM, [ascorbate] = 100 μM. ROHs were added before the initiation with the initial concentrations of 0.13, 0.25, 0.5, 1.0 and 2.0 μM, respectively, as shown in the figure. Values represent mean ± S.E. of three experiments.

effect of ROHs with the endogenous antioxidants in the rat liver microsomes, that is, the inhibition period of the microsomes was increased appreciably longer than the sum of the intrinsic t_{inh} of the native microsomes and the t_{inh} induced by ROHs when they were used alone (compare Fig. 1A and B). The increase of the inhibition time follows the sequence of 3,4-DHS>4,4′-DHS>resveratrol>4-HS ~ 3,5-DHS, same to the sequence of the decrease of the oxygen uptake rate mentioned above.

3.2. Inhibition of AAPH-induced microsomal peroxidation by ROHs as studied by TBARS formation

Fig. 2 shows the TBARS formation during the AAPH-induced peroxidation of rat liver microsomes. Similar to the oxygen uptake experiments mentioned in the previous section, the formation of TBARS was inhibited for a short period of time upon AAPH initiation due to the endogenous antioxidants in the microsomes. It is seen that the rate of TBARS formation increased with the concentration of AAPH used and the inhibition period correlated approximately inversely to the concentration of AAPH as expected. Fig. 3 shows the inhibition of TBARS formation by resveratrol. It is clearly seen that resveratrol significantly suppressed rate of TBARS formation and increased the inhibition period in a dose-dependent manner. Other ROHs showed the same behavior and a set of representative results is shown in Fig. 4. All of these ROHs diminished the rate of TBARS formation and increased the inhibition period. The activity sequence is 3,4-DHS>4,4′-DHS>resveratrol>4-HS>3,5-DHS, similar to that observed in the oxygen uptake assay mentioned above.

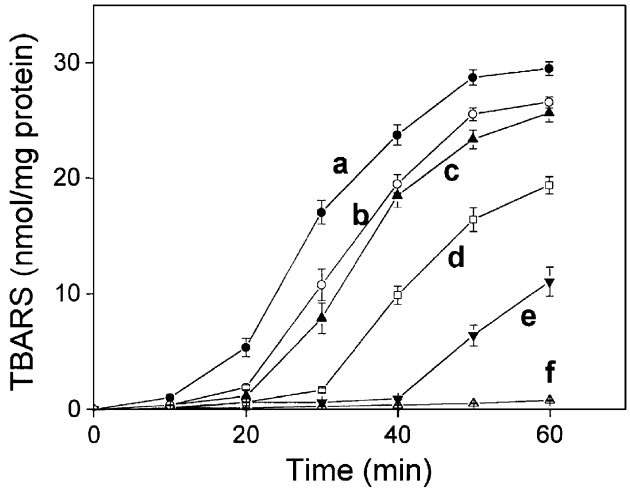


Fig. 6. Inhibition of TBARS formation during the Fe^{2+}/ascorbate-induced peroxidation of rat liver microsomes by ROHs at 37 °C. The rat liver microsomes were suspended in 0.1 M potassium phosphate buffer (pH 7.5) at a protein concentration of 0.3 mg/ml. [$FeSO_4$] = 10 μM; [ascorbate] = 100 μM; $[ROHs]_0$ = 0.5 μM. (a) Native microsomes; (b) 3,5-DHS; (c) 4-HS; (d) resveratrol; (e) 4,4′-DHS; (f) 3,4-DHS. Values represent mean ± S.E. of three experiments.

3.3. The antioxidant effect of resveratrol and its analogues on Fe^{2+}/ascorbate-induced rat liver microsomal peroxidation

Iron (Fe^{2+} plus a reducing reagent) is an extensively used system for generating hydroxyl radicals to induce lipid peroxidation that can be measured by the TBARS assay [24]. In the present study, we used Fe^{2+}/ascorbate to induce peroxidation of rat liver microsomes to investigate the antioxidant effect of resveratrol and its analogues (ROHs). It was found that ROHs preincubated with rat liver microsomes inhibited TBARS production in a dose-dependent and time-dependent manner (Figs. 5 and 6). The antioxidant activity also follows the sequence of 3,4-DHS>4,4′-DHS>resveratrol>4-HS>3,5-DHS.

4. Discussion

The first event of lipid peroxidation is oxygen absorption and a final product of the peroxidation is TBARS [20]. In the present work, both the indexes were used to evaluate the antioxidative activity of resveratrol and its analogue (ROHs) to find the structural determinant responsible for the antioxidant activity in vitro. In addition, two different initiating assays, that is, AAPH which produces alkyl radicals followed by fast reaction with oxygen to give initiating alkyl peroxyl radicals (Eqs. (1) and (2)) [14,25] and Fe^{2+}/ascorbate which produces hydrogen peroxide and the latter generates hydroxyl radicals by Fenton reaction (Eq. (3)) [26], were used to initiate the microsomal peroxidation (Eqs. (4)–(7)).

$$AAPH \rightarrow 2R^{\cdot} + N_2 \quad (1)$$

$$R^{\cdot} + O_2 \rightarrow ROO^{\cdot} \quad (2)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{-} + OH^{\cdot} \quad (3)$$

$$ROO^{\cdot} + LH \rightarrow ROOH + L^{\cdot} \quad (4)$$

$$OH^{\cdot} + LH \rightarrow H_2O + L^{\cdot} \quad (5)$$

$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot} \quad (6)$$

$$LOO^{\cdot} + LH \rightarrow LOOH + L^{\cdot} \quad (7)$$

where $R^{\cdot}$ is the alkyl radical generated from the thermal decomposition of AAPH and LH represents a lipid molecule with an abstractable hydrogen, that is, polyunsaturated fatty acids in microsomes. In the presence of an antioxidant molecule, AH, either the initiating peroxyl radical, hydroxyl radical and/or the propagating lipid peroxyl radical can be trapped and a new antioxidant radical, $A^{\cdot}$, produced (Eqs. (8)–(10)). If the $A^{\cdot}$ is a stabilized radical (e.g., α-tocopheroxyl radical or ascorbate radical) which can promote the rate-limiting hydrogen abstraction reactions (Eqs. (8)–(10)) and

undergo fast termination reactions (e.g., Eqs. (11) and (12)), the peroxidation would be inhibited.

$$ROO^{\cdot} + AH \rightarrow ROOH + A^{\cdot} \quad (8)$$

$$OH^{\cdot} + AH \rightarrow H_2O + A^{\cdot} \quad (9)$$

$$LOO^{\cdot} + AH \rightarrow LOOH + A^{\cdot} \quad (10)$$

$$A^{\cdot} + LOO^{\cdot} \rightarrow LOOA \quad (11)$$

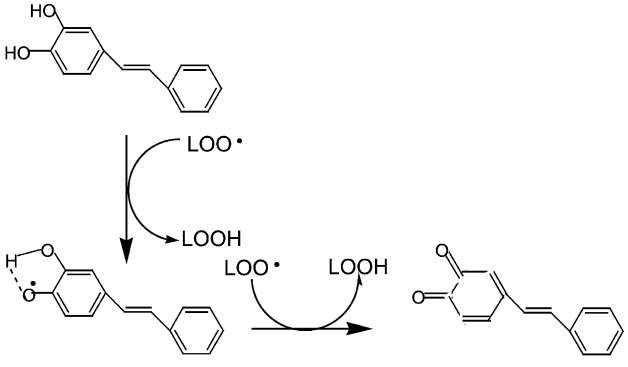
$$2A^{\cdot} \rightarrow A - A + \text{others} \quad (12)$$

The rate of the chain propagation, R_p for Eq. (7), the inhibited rate of propagation by antioxidants, R_{inh} for Eq. (10), and the inhibition period, t_{inh}, can be easily obtained from the oxygen uptake experiment (Fig. 1) and are listed in Table 1. The fact that no new inhibition period could be observed when ROHs were added after the intrinsic inhibition period (Fig. 1A) suggests that the ROHs react with the initiating radicals in the bulk water phase (Eq. (8)), hence diminish the effective rate of initiation, which in turn, decreases the rate of propagation [14]. In this case, the antioxidant activity of ROHs can be expressed by (R_{inh}/R_p)100, designating the percentage inhibition of the peroxidation which is independent on the reaction time.

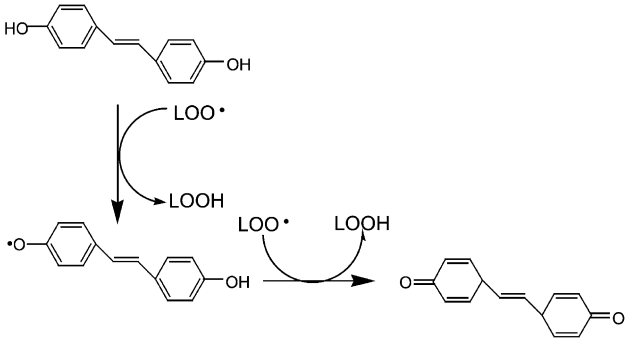
On the other hand, if the ROHs were added before the AAPH initiation, the inhibition period of the native microsomes was markedly prolonged (Fig. 1B). The synergistic antioxidant efficiency, SE %, is generally expressed by Eq. (13) [14] where AH is the endogenous antioxidant in the microsomes. The results are listed in Table 1. In this case, the antioxidant activity of ROHs can be evaluated by comparing their t_{inh} and/or SE %.

$$SE\ \% = [t_{inh}(ROH + AH) - t_{inh}(AH) - t_{inh}(ROH)] / [t_{inh}(ROH) + t_{inh}(AH)]100\% \quad (13)$$

The antioxidant synergism of TOH with co-antioxidants such as L-ascorbic acid (vitamin C) [27], β-carotene [14d,21,28] and green tea polyphenols [14] has been extensively studied and rationalized to be due to the regeneration of TOH by these co-antioxidants. We have also proved recently by using stopped-flow electron paramagnetic resonance (EPR) spectroscopy that resveratrol and 3,4-DHS could reduce α-tocopheroxyl radical ($TO^{\cdot}$) to regenerate TOH (Eq. (14)) with bimolecular rate constants of 0.23×10^2 and $3.0 \times 10^2\ M^{-1}\ s^{-1}$, respectively, in cetyl trimethylammonium bromide (CTAB) micelles [15]. Therefore, it is reasonable to suggest that the same mechanism might be involved in the synergistic antioxidation reaction of ROHs with the endogenous TOH in rat liver microsomes. The concentration of TOH in the microsomes was determined to be 0.3 nmol/mg protein.



Scheme 1. Mechanism of 3,4-DHS inhibited peroxidation.



Scheme 2. Mechanism of 4,4′-DHS inhibited peroxidation.

$$TO^{\cdot} + ROH \rightarrow TOH + RO^{\cdot} \quad (14)$$

On the other hand, the prolonged inhibition period in the presence of ROHs might also be caused by the reduced initiation rate due to the ROHs. Since the inhibition period is inversely proportional to the initial concentration of AAPH [14], that is, the rate of initiation, reduced initiation rate as shown in Fig. 1A should prolong the inhibition period. It can be seen from Table 1 that the ratio of t_{inh}(AH)/t_{inh}(ROH+AH) follows the same sequence of R_{inh}/R_p, suggesting a correlation between the antioxidant synergism and the rate of effective initiation. However, the appreciable smaller value of t_{inh}(AH)/t_{inh}(ROH+AH) than R_{inh}/R_p in case of 3,4-DHS, 4,4′-DHS and resveratrol implies that the reduced rate of initiation is not the only reason for the antioxidant synergism and the TOH regeneration reaction (Eq. (14)) might also contribute to the antioxidant synergism of these compounds.

It is clearly seen by comparison of Fig. 1 with Figs. 4–6 that the antioxidant activity of ROHs follows the same sequence in spite of the activity being monitored by oxygen uptake or by TBARS formation, and in spite of the peroxidation being initiated by peroxyl radicals (generated by AAPH) or by hydroxyl radicals (generated by Fe^{2+}/ascorbate). 3,4-DHS, which bears an *ortho*-diphenoxyl functionality, is remarkably active than resveratrol and other ROHs. This is understood in view of the antioxidation mechanism of phenolic antioxidants as exemplified in Scheme 1 [15].

The *ortho*-hydroxyl would make the oxidation intermediate, *ortho*-hydroxyl phenoxyl radical, more stable due to the intramolecular hydrogen bonding interaction as evidenced recently from both experiments [29] and theoretical calculations [30]. The theoretical calculation showed that the hydrogen bond in *ortho*-OH phenoxyl radical is ca. 4 kcal/mol stronger than that in the parent molecule catechol and the bond dissociation energy (BDE) of catechol is 9.1 kcal/mol lower than that of phenol and 8.8 kcal/mol lower than that of resorcinol [30]. In addition, *ortho*-OH phenoxyl radical shall be easier further oxidized to form the final product *ortho*-quinone [29] (Scheme 1). The 4′-OH group also enhanced the activity since the 4′-OH group can stabilize the phenoxyl radical intermediate by resonance through the *trans*-double bond and be further oxidized to the *para*-quinone (Scheme 2). It has recently been proved that the 4′-OH is more active than the *meta*-dihydroxyl groups in resveratrol [4a,30,31].

5. Conclusions

Resveratrol and its analogues, that is, 3,4-DHS, 4,4′-DHS, 4-HS and 3,5-DHS, are effective antioxidants against both AAPH- and iron-induced peroxidation of rat liver microsomes. The antioxidant mechanism may involve trapping the initiating peroxyl radicals and/or hydroxyl radicals and reducing α-tocopheroxyl radical ($TO^{\cdot}$) to regenerate the endogenous TOH. The observation that *trans*-stilbene compounds bearing *ortho*-diphenoxyl or *para*-diphenoxyl functionalities possess remarkably higher antioxidant activity than resveratrol gives us useful information for antioxidant drug design.

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